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12. (Amended) The method of claim 10, wherein said protein of interest comprises a toxin.

14. (Amended) A method for producing a protein of interest in a specific organ, said method comprising contacting cells of said organ with a masked expression cassette comprising a double stranded nucleic acid molecule, wherein a first strand comprises an RNA sequence which codes for said protein of interest linked downstream of a flanking sequence, and a translation initiation site operably inserted upstream of the RNA sequence; and

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a second strand bound to the flanking sequence, wherein said second strand comprises a polynucleotide corresponding to said first strand and to a target molecule specific to said organ.

Support for these amendments can be found in the claims as originally filed, as well as in the specification (*see* page 6 and Figure 7). No new matter has been incorporated, thereby.

REMARKS

Status of the Claims

Claims 1, 3, 4, 5, 10, 12, and 14 have been amended without prejudice as noted elsewhere. No new matter has been incorporated thereby. Claims 1-15 are pending. Support for these new claims and amendments can be found in the specification and claims as originally filed. No new matter is added by way of claim amendment. Applicant respectfully submits the claims as amended are enabled by the specification.

The Examiner's comments in the Office Action are addressed below in the order set forth therein.

Claims Rejection Under 35 U.S.C. § 112, second paragraph

Claims 1, 5, 10, and 14 are rejected under 35 U.S.C. §112, second paragraph on the basis that there is no antecedent basis for "a second antisense strand" or "said strand." These claims have been amended without prejudice to recite "a second strand bound to the flanking sequence, wherein said second strand comprises a polynucleotide corresponding to said first strand and to a target molecule." Applicant submits that this amendment obviates the Examiner's rejection and respectfully requests that it be withdrawn.

The Office Action also states that the meaning of the term "corresponds" in these claims is unclear. The claims have been amended to recite "a polynucleotide corresponding to said first strand and to a target molecule." The term "corresponding" is defined in the specification to mean "at least partially complementary." Read in light of the specification, the scope of the claims is clear. Applicant respectfully requests that this rejection be withdrawn.

It is stated in the Office Action that, in claim 10, it is unclear whether the term "target" on line 9 is related to the term "target" on line 2. Claim 10 has been amended without prejudice to recite "the target" in place of "a target" to clarify that they are related. Applicant respectfully submits that the Examiner's rejection is obviated by this amendment and requests that it be withdrawn.

Applicant has amended claim 10 without prejudice, inserting a "to" between "bound" and "the," and corrected "marked" to read "masked." Applicant submits that these amendments are fully responsive to the rejection in the Office Action and respectfully requests that they be withdrawn.

Applicant has also amended claims 3 and 12 without prejudice to recite "wherein said protein of interest *comprises* a toxin." Applicant respectfully requests that this rejection be withdrawn.

Applicant has also amended claim 4 without prejudice to recite "wherein said target is unique to neoplastic cells" and submits that this obviates the Examiner's rejection with respect to claim 4.

Claims 2, 6-9, 11, and 13 were also rejected as depending from rejected claims. In light of the amendments described above, Applicant respectfully requests that the rejection of claims 2, 6-9, 11, and 13 be withdrawn, as well.

Claims Rejection Under 35 U.S.C. §112, first paragraph

Claims 1-15 under 35 U.S.C. §112, first paragraph, on the basis that Applicant has not adequately described the claimed invention. The rejection is respectfully traversed.

The Office Action states that Applicant does not describe how one would maintain the masked expression cassette construct. However, the drawings and specification clearly show that the construct is formed by hydrogen bonding, i.e., "hybridization," between complementary strands (Figures 1-6 and specification, page 6, paragraph 3, page 17, paragraph 1 & 2). It is asserted in the Office Action that "[i]t is well known that hydrogen bonding is a transient association with many factors especially in a cellular environment that affect such associations." However, the Examiner does not provide specific examples of these factors, nor are any references provided to indicate that the construct described in the figures and specification would likely be unstable. Applicant submits that, while hydrogen bonding is not as stable as a covalent bond, it is well known that hybridization between two complementary strands is a stable interaction. Nonetheless, it is also known in the art that strand displacement reactions between similar nucleic acid strands, where one strand is displaced in favor of a more complementary strand, take place even at 37°C. *See* Nedbal and Sczakiel, *Nucleic Acids Res.* (1996) 24:4395.

These are well-established scientific principles. The Examiner is further referred to Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor, New York). Thus, the claimed invention satisfies the written description requirement of 35 U.S.C. §112, first paragraph. The Application provides a description of the concept (specification, pages 5-10), as well as many examples describing embodiments of the claimed invention (pages 16-20). For example, specific examples of flanking sequences, and the corresponding antisense strand, are provided (page 19, paragraphs 1 & 2). As the invention is based on established

scientific principles of polynucleotide association kinetics and hybridization, Applicant respectfully requests that the rejection be withdrawn.

Claims 1-15 are also rejected under 35 U.S.C. §112, first paragraph, on the basis that one of skill in the art would not be able to use the invention. (The Office Action notes that Applicant has enabled one of skill in the art to make the invention.) This rejection is respectfully traversed.

With respect to maintaining or unmasking the expression cassette, Applicant submits that, as explained above, the construct is maintained by hydrogen bonds between the bases of complementary strands. Specifically, this interaction occurs between the flaking sequence and the antisense oligonucleotide. The robustness of hybridization between two DNA stands or the strength of the nucleotide association depends upon the extent of homology between the sequences. The more complementary or homologous the sequences, the stronger the association due to an increase in hydrogen bonding between the sequences. The specification further explains that the cassette becomes unmasked when in the presence of the target molecule as the antisense oligonucleotide preferentially binds to the target molecule (specification, pages 9-10). As set forth above, it is known in the art that strand displacement reactions can take place even at 37°C. Strand displacement occurs because the target molecule shares greater homology with the antisense oligonucleotide than the flanking sequence. *See* Nedbal and Sczakiel.

The Office Action also contends that no guidance is provided for how the cassette becomes unmasked in circumstances such as "regulating the expression of a gene to be expressed at a certain time" or "where it is desirable to regulate gene expression." The gist of the current claims is not to regulate expression either in a temporal or tissue-specific manner. Rather, expression is controlled by the presence, or absence, of the target molecule which is based on established principles of DNA-DNA/DNA-RNA association.

The Office Action also states that the specification does not "teach how to obtain sufficient levels of expression of the protein of interest in the presence of a target molecule such that one could provide a therapy or specifically regulate expression of the protein of interest."

This suggests that method of therapy is being claimed. This is not the case, however. What is claimed is the described masked expression cassette, methods of producing a protein of interest in cells and organs, and methods of inhibiting the growth of neoplastic cells. With respect to regulation of the protein of interest, as the figures and the specification describe, the expression cassette will become unmasked in the presence of the target molecule due to the greater degree of complementarity or homology between the target and the oligonucleotide, exposing the ribosome binding site and allowing ribosomal binding, thus allowing expression of the protein of interest encoded by the cassette (specification, pages 16-17). Thus, for each cassette exposed due to the presence of a target molecule, a cassette RNA will become available for ribosomal binding and subsequent translation. Accordingly, regulation of the expression of the protein of interest is controlled by the availability of the target nucleic acid, by the number of masked expression cassettes, and the stability of the unmasked RNA. In the case where the protein of interest is a toxin, even limited expression will result in the desired outcome—cell death. Given this guidance, one of skill in the art could vary the identified parameters and practice the invention without undue experimentation.

The Office Action also cites extensively to three review articles that discuss the difficulties of gene therapy and antisense therapies. Specifically, the Office Action cites Branch *TIBS* (1998) 23:45-51 for the proposition that antisense strategies have proven unpredictable. For instance, Branch points out that, although they are designed to "bind to and inhibit target RNAs through complementary Watson-Crick base pairing," antisense molecules frequently have "non-antisense" effects (page 45, columns 1 & 2). For instance, in some initially promising studies, the beneficial anti-proliferative effects first thought to be due to specific binding by the antisense oligodeoxynucleotide were later discovered to be likely due to cytotoxic oligodeoxynucleotide breakdown products (page 46, column 3).

The unpredictability highlighted in Branch is only generally relevant to the present invention, however. While antisense technology is involved in the present invention, the mechanism of action of the claimed invention is not through "bind[ing] and inhibit[ing] target

RNAs through complementary Watson-Crick base pairing." Rather, once the expression cassette is unmasked, the mechanism of action is through the expression of a protein of interest encoded by the cassette (specification, pages 4, 6-7). It is the protein which has the effect on the cell which contains the target RNA. According to Branch, antisense technology, which relies on binding by the antisense molecule to cause destruction/inhibition of the offending RNA target, is problematic because "to be effective, a high dosage of antisense molecules have to be delivered to each cell" (specification, page 16). The present invention allows the dosage of the antisense molecule to be much lower because it is the protein of interest which causes the effect, not the antisense molecule.

Similarly, the other articles cited in the Office Action are of only general relevance to the present invention. For example, Anderson, *Nature* (1998) 392:25-30, mainly discusses the difficulties associated with gene therapy involving viral based vectors. In fact, Anderson notes that synthetic gene-delivery systems avoid toxic effects engendered by active viral particles and, further, that manufacturing a synthetic product should be less complex than using tissue culture cells as bioreactors to produce viral vectors (page 28, column 2, paragraph 3). Thus, Anderson highlights difficulties that Applicant's invention actually avoids because it does not involve a viral-based vector. Verma *et al.* is similarly focused on viral-based vectors and of little relevance to Applicant's invention.

For all these reasons, the pending claims are fully enabled and the rejection should be withdrawn.

Consideration Of Previously Submitted Information Disclosure Statement

It is noted that an initialed copy of the PTO Form 1449 that was submitted with Applicants' Information Disclosure Statement filed December 20, 1999 has not been returned to Applicants' representative with the Office Action. Accordingly, it is requested that an initialed copy of the Form 1449 be forwarded to the undersigned with the next communication from the PTO. In order to facilitate review of the references by the Examiner, a copy of the Information

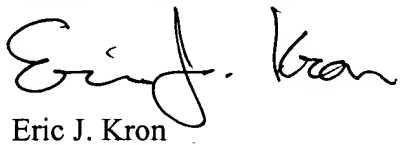
Disclosure Statement and the Form 1449 are attached hereto. Copies of the cited references were provided at the time of filling the original Information Disclosure Statement, and, therefore, no additional copies of the references are submitted herewith. Applicants will be pleased to provide additional copies of the references upon the Examiner's request if it proves difficult to locate the original references.

CONCLUSION

In view of the aforementioned amendments and remarks, Applicant respectfully submits that the rejections of the claims under 35 U.S.C. §112, first and second paragraph, are overcome and that this application is now in condition for allowance. Early notice to this effect is solicited.

It is not believed that extensions of time or fees for net addition of claims are required, beyond those that may otherwise be provided for in documents accompanying this paper. However, in the event that additional extensions of time are necessary to allow consideration of this paper, such extensions are hereby petitioned under 37 CFR §1.136(a), and any fee required therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. 16-0605.

Respectfully submitted,



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CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, Washington, DC 20231, on April 4, 2001.

Nora C. Martinez

Version with Markings to Show Changes Made:

In The Specification:

Please amend the specification, as follows:

Please replace page 5, "Brief Description of the Drawings" Section, paragraph 7, with the following paragraph:

Figure 7 provides an example of a construct for production of the sense strand of the targeted cassette. The Kozak sequence is also shown (SEQ ID NO:17).

Please replace page 8, paragraph 4, with the following paragraph:

The translation initiation site can be inserted upstream of the sequence corresponding to the gene of interest. Kozak sequences can be designed that can initiate translation in all three reading frames. See, for example, Murphy and Efstratiadias (1987) *Proc. Natl. Acad. Sci. USA, 84*:8277-8281. Generally, the Kozak sequence will comprise the consensus sequence recognized for initiation in higher eukaryotes. Such consensus sequence is GCCGCC$^{A}_{G}$CCAUGG (SEQ ID NO:18). This consensus sequence is repeated several times within the Kozak sequence to provide for the initiation of translation in all three reading frames.

Please replace page 8, paragraph 6, with the following paragraph:

It is recognized that a prokaryotic translation initiation site may also be used when appropriate; for example, when targeting a prokaryote. Such sequences include the Shine-Dalgarno sequence (UAAGGAGG (SEQ ID NO:19)), typically 5-10 bases upstream of the initiator AUG.

In The Claims:

Please amend claims 1, 3, 4, 5, 10, 12, and 14, as follows:

1. (Amended) A masked expression cassette comprising a double stranded nucleic acid molecule wherein a first strand comprises an RNA sequence which codes for a protein of interest linked downstream of a flanking sequence, and a translation initiation site operably inserted upstream of the RNA sequence; and,

a second [antisense] strand bound to the flanking sequence, wherein said second strand [corresponds to] comprises [an antisense oligonucleotide] a polynucleotide corresponding to said first strand and to a target molecule.

3. (Amended) The cassette of claim 1, wherein said protein of interest [encodes] comprises a toxin.

4. (Amended) The cassette of claim 1, wherein said target [comprises an oligonucleotide which] is unique to neoplastic cells.

5. (Amended) A method for inhibiting the growth of neoplastic cells, said method comprising contacting said cells with a masked expression cassette comprising a double stranded nucleic acid molecule;

wherein a first strand comprises an RNA sequence which codes for a protein of interest linked downstream of a flanking sequence, and a translation initiation site operably inserted upstream of the RNA sequence; and,

a second [antisense] strand bound to the flanking sequence, wherein said second strand [corresponds to] comprises [an antisense oligonucleotide] a polynucleotide corresponding to said first strand and to a target molecule.

10. (Amended) A method for controlling the expression of a protein of interest in the presence of a target molecule, said method comprising contacting a cell comprising the target molecule with a [marked] masked expression cassette comprising a double stranded nucleic acid molecule, wherein a first strand comprises an RNA sequence which codes for a protein of interest linked downstream of a flanking sequence, and a translation initiation site operably inserted upstream of the RNA sequence; and[,]

a second [antisense] strand bound to the flanking sequence wherein said second strand [corresponds to] comprises [an antisense oligonucleotide] a polynucleotide corresponding to said first strand and to [a] the target molecule.

12. (Amended) The method of claim 10, wherein said protein of interest [encodes] comprises a toxin.

14. (Amended) A method for producing a protein of interest in a specific organ, said method comprising contacting cells of said organ with a masked expression cassette comprising a double stranded nucleic acid molecule, wherein a first strand comprises an RNA sequence which codes for said protein of interest linked downstream of a flanking sequence, and a translation initiation site operably inserted upstream of the RNA sequence; and[,]

a second [antisense] strand bound to the flanking sequence, wherein said [antisense] second strand [corresponds] comprises a polynucleotide corresponding to said first strand and to a target molecule specific to said organ.